Analysis of methane production pathways in a riparian wetland of a temperate forest catchment, using δ_13 C of pore water CH_4 and CO_2

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Analysis of methane production pathways in a riparian wetland of a temperate forest catchment, using $\delta^{13}C$ of pore water CH_4 and CO_2

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[1] To clarify how hydrological processes affect biogenic methane (CH_4) production and emission from soil surfaces, we analyzed the $\delta^{13}C$ of CH_4 and CO_2 and chemical constituents dissolved in groundwater at a wetland in the headwater catchment of a temperate forest in Japan. We estimated the contribution of acetate fermentation using the $\delta^{13}C$ isotope mass balance of dissolved CH_4 and CO_2. CH_4 production pathways (e.g., acetate fermentation and carbonate reduction) changed temporally and spatially with hydrologically controlled redox conditions. The proportion of methanogenesis attributable to acetate fermentation usually decreased with temperature, suggesting that carbonate reduction dominated under conditions of high CO_2 concentration. In particular, the groundwater table and summer temperatures were key controlling factors in the interannual and intra-annual changes in CH_4 production pathways, controlling oxygen supply and consumption and, therefore, redox conditions in the soil. Under high temperature and high water table conditions during summer, the soil was strongly reduced and the proportion of carbonate reduction increased. Acetate fermentation also increased episodically, resulting in sporadic increases in $\delta^{13}C$-CH_4. The calculated acetate contribution obviously decreased in periods of low water table and high temperature when the soil surface was relatively oxic, implying deactivation of acetoclastic methanogenesis under oxic conditions. Thus, hydrological processes control the supply of these electron donors and acceptors and therefore play an important role in determining the relative proportions of CH_4-producing pathways. Our results also indicate that an increase in acetate contribution under highly reducing conditions stimulates CH_4 production and emission from the soil surface.

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1. Introduction

[2] Methane (CH_4) is a key greenhouse gas, and its infrared radiative heating effect is 26 times greater than that of carbon dioxide on a mole-per-mole basis [*Lelieveld and Crutzen*, 1992]. Soil functions as both a main source and a main sink of CH_4. In anoxic environments such as wetland soils, CH_4 is produced by methanogenic bacteria that are active only under anoxic and strongly reducing conditions [*Takai*, 1970; *Schütz et al.*, 1989]. In contrast, CH_4 is usually oxidized by methanotrophic bacteria in oxic soils. Over the last 150 years, the mixing ratio of CH_4 in the atmosphere has more than doubled [*Etheridge et al.*, 1998], and improved estimates of the strength of each source and sink are of high importance. However, much uncertainty regarding the CH_4 production and consumption mechanisms in soils still leads to uncertainty in estimating the levels of atmospheric CH_4.

[3] Because biospheric sources of CH_4 are highly variable, stable isotope ratios of CH_4 have been used to constrain the global CH_4 budget, as microbe-produced CH_4 has a significantly different isotopic signal than CH_4 from other sources [*Whiticar*, 1999]. In particular, the ^{13}C compositions of CH_4 in background tropospheric air and of the major CH_4 sources have added further constraint to the individual CH_4 source strengths by isotope mass balance using the $\delta^{13}C$ value of each source [*Stevens and Rust*, 1982; *Cicerone and Oremland*, 1988; *Stevens and Engelkemeir*, 1988; *Whalen et al.*, 1989; *Quay et al.*, 1991; *Lowe et al.*, 1994; *Gupta et al.*, 1996; *Bräunlich et al.*, 2001; *Fletcher et al.*, 2004] and have revealed that 70–80% of atmospheric CH_4 is of biogenic origin, with natural wetlands as the largest source [*Bartlett and Harriss*, 1993; *Khalil and Shearer*, 1993]. However, estimating the representative $\delta^{13}C$-CH_4 of each source

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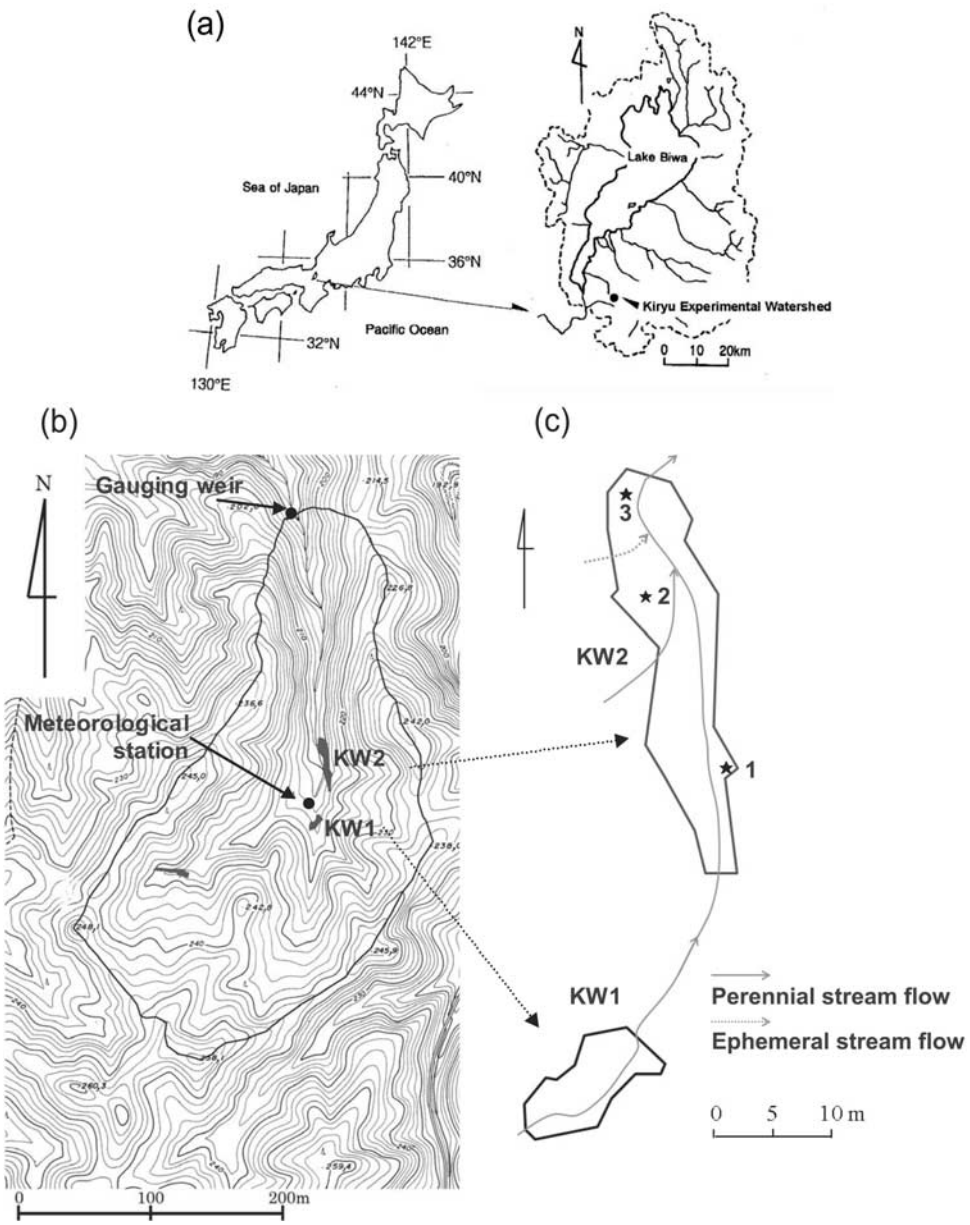

Figure 1. (a) Location of KEW. (b) Topographic map of KEW and the locations of wetlands. Shading indicates riparian wetland areas. (c) Locations of the observation plots. Each number indicates an observation plot (1: KW2-edge, 2: KW2-center, 3: KW2-downstream).

remains challenging because $\delta^{13}C$-CH_4 values are highly variable, especially in rice paddies and wetlands [*Quay et al.*, 1991], reflecting the multiple processes involved in CH_4 production and consumption in these ecosystems. For example, methanogenesis from carbonate results in a larger fractionation against ^{13}C and, thus, lower $\delta^{13}C$-CH_4 values than methanogenesis from acetate [*Games et al.*, 1978; *Krzycki et al.*, 1987; *Gelwicks et al.*, 1994]. However, previous studies have shown that the fractionation factors vary with site and conditions (e.g., reviewed by *Conrad* [2005]). More data under various environmental conditions are required for the explicit determination of fractionation factors.

[4] Among the various CH_4 sinks and sources, forests are assumed to be a major sink of atmospheric CH_4 by microbial oxidation in aerobic soils [*Reeburgh et al.*, 1993; *IPCC*, 2001], However, *Itoh et al.* [2005, 2007] suggested that wet riparian areas in forests can function as 'hot spots' of CH_4 emission and that these 'hot spots' can also significantly affect the total budget of trace gas emissions on larger scales, such as whole forest ecosystems. Soil hydrological conditions in forest catchments are spatially variable. Riparian wetlands in small headwater catchments are characterized by high CH_4 production, and production and emission rates are strongly affected by changes in hydrological processes and temperature [*Itoh et al.*, 2007]. This hydrological variability can also affect CH_4 production pathways that mainly consist of acetate fermentation [*Zeikus et al.*, 1975] and carbonate reduction [*Takai*, 1970; *Crill and Martens*, 1986; *Martens et al.*, 1986; *Burke et al.*, 1988; *Schütz et al.*, 1989]. Redox conditions in such wetland soils

Table 1. Mean Annual Air Temperature and Annual and Summer Precipitation in the Kiryu Experimental Watershed[a]

	2003	2004	2005	1996–2005
Mean annual air temperature (°C)	12.9	13.9	13.0	13.2[b]
Annual precipitation (mm $year^{-1}$)	1946.8	1796.8	1150.5	1574.1
Summer precipitation (mm)[c]	804.0	500.3	474.0	550.6

[a]Data from *Itoh et al.* [2007].
[b]Average of values obtained from 2000 to 2005.
[c]From June to August.

change on a shorter temporal scale with hydrological conditions (precipitation patterns and water movement in soil [*Mitsch and Gosselink*, 2000; *Itoh et al.*, 2007]) than in ombrotrophic wetlands, where most previous work has been conducted. CH_4 production pathways can change drastically on small temporal and spatial scales in such riparian wetlands. Thus, for a more reliable estimate of the CH_4 budget on a larger scale, such as an entire forest catchment, an understanding of CH_4 production mechanisms, including production pathways, is strongly required.

[5] In this study, we used the $\delta^{13}C$ isotope mass balance of pore water CH_4 and CO_2 [*Sugimoto and Wada*, 1993] to determine temporal and spatial changes in CH_4 production pathways and to understand what processes control pathway changes in riparian wetlands. In addition, we considered the effects of climate differences, such as characteristic precipitation patterns in Asian monsoon climates, on CH_4 production pathways.

2. Materials and Methods

2.1. Site Description and Hydrological and Biogeochemical Features

[6] We studied forested wetlands in the Kiryu Experimental Watershed (KEW; 35°N, 136°E; 190–255 m above sea level; 5.99 ha), located in southeastern Shiga Prefecture, central Japan (Figure 1). The KEW comprises about 99.3% forest floor, with 0.67% (400.6 m^2) distinct wetland riparian zones. The wetland studied here, Kiryu Wetland 2 (KW2), is located upstream of a check dam constructed across the mainstream of the watershed about 100 years ago to prevent soil erosion. There are other natural wetlands in KEW, but all are located in riparian zones along streams. The wetland soils are either always submerged or periodically submerged. The entire watershed is on a base of weathered granitic rock with an abundance of albite. In the 1960s, Japanese cypress (*Chamaecyparis obtusa*) was planted on the hillslope over the watershed. Although several tree species (*Eurya japonica*, *Alnus japonica*, *Clethra barvinervis*, *Evodiopanax innovans*, and *Rhus trichocarpa*) and sphagnum grow in and around the wetlands, vegetation in the wetlands is sparse, probably because of occasional sediment transport with surface flow.

[7] Precipitation was measured at a meteorological station within the watershed. Mean annual air temperature and precipitation were recorded during the observation period (Table 1). Surface soil temperatures at depths of 0.02 and 0.10 m were also continuously monitored (Figure 2a) at each groundwater-sampling plot. The water levels in KW2 were measured in wells installed at each groundwater-sampling plot. We also installed a capacitance water level sensor and data recorder (CR-10x, Campbell, USA) 0.50 m from the KW2-downstream plot.

[8] The study site is affected by the Asian monsoon system, which usually results in a summer rainy season. Variation was observed in summer (June, July, and August) precipitation; a large amount of precipitation was observed in 2003, and less in 2004 and 2005 (Table 1 and Figure 2b). Summer precipitation accounted for 146.0, 90.9, and 86.1% of the mean annual precipitation (1574.1 mm for 1996–2005) in 2003, 2004, and 2005, respectively.

[9] Strongly reducing conditions (e.g., activation in denitrification and manganese-, iron-, and sulfate-reductions) were formed in high temperature periods in all three sampling plots [*Itoh et al.*, 2007]. However, relatively dissolved oxygen (DO)-rich stream and subsurface flow from the hillslope due to heavy precipitation induced more oxic conditions than usual in the surface and bottom layers of wetlands in 2003. In contrast, a decrease in the water table formed oxic conditions in the surface soil layer in summer 2005. In these summers, the CH_4 concentration in the surface soil was much lower than in the summer of 2004, when the water table and soil temperature were high. These changes in redox conditions, depending on hydrologic conditions, strongly affect CH_4 production in the soil and CH_4 emission from the soil surface [*Itoh et al.*, 2007].

2.2. Groundwater Collection and Analysis

[10] Pore water samples were collected vertically to measure the concentration (of CH_4 and CO_2) and isotopic composition of $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 and water chemistry in each of three observation plots, KW2-edge (0.55 m soil depth), KW2-center (1.77 m), and KW2-downstream (1.38 m; Figure 1c). KW2-edge was near the hillslope and KW2-downstream was along a perennial stream (Figure 1c). The surface soils of all three sampling plots were silty with much undecomposed litter. We used double-walled pore water samplers [*Itoh et al.*, 2007] which collected pore water without degassing and high decompression. The pore water samplers were placed at KW2-edge (at soil depths of 0.10 and 0.25 m), KW2-center, and KW2-downstream (at 0.10, 0.20, 0.30, 0.50, and 0.70 m). Pore water samples were injected into 20-, 30-, or 50-mL pre-evacuated vials for the measurement of dissolved CH_4 and CO_2 concentrations and their carbon isotope ratios without exposure to the atmosphere, and into plastic bottles for other chemical analyses; the vials and plastic bottles were stored in a cooler (around 4°C) in the field. Surface water (KW2-edge and KW2-center) and stream water (KW2-downstream) were also sampled.

[11] In situ measurements, including pH and electrical conductivity (EC), and laboratory measurements of dissolved components including DO, CH_4, and CO_2 were conducted. Detailed information on the methods and water chemical constituent results were given by *Itoh et al.* [2007].

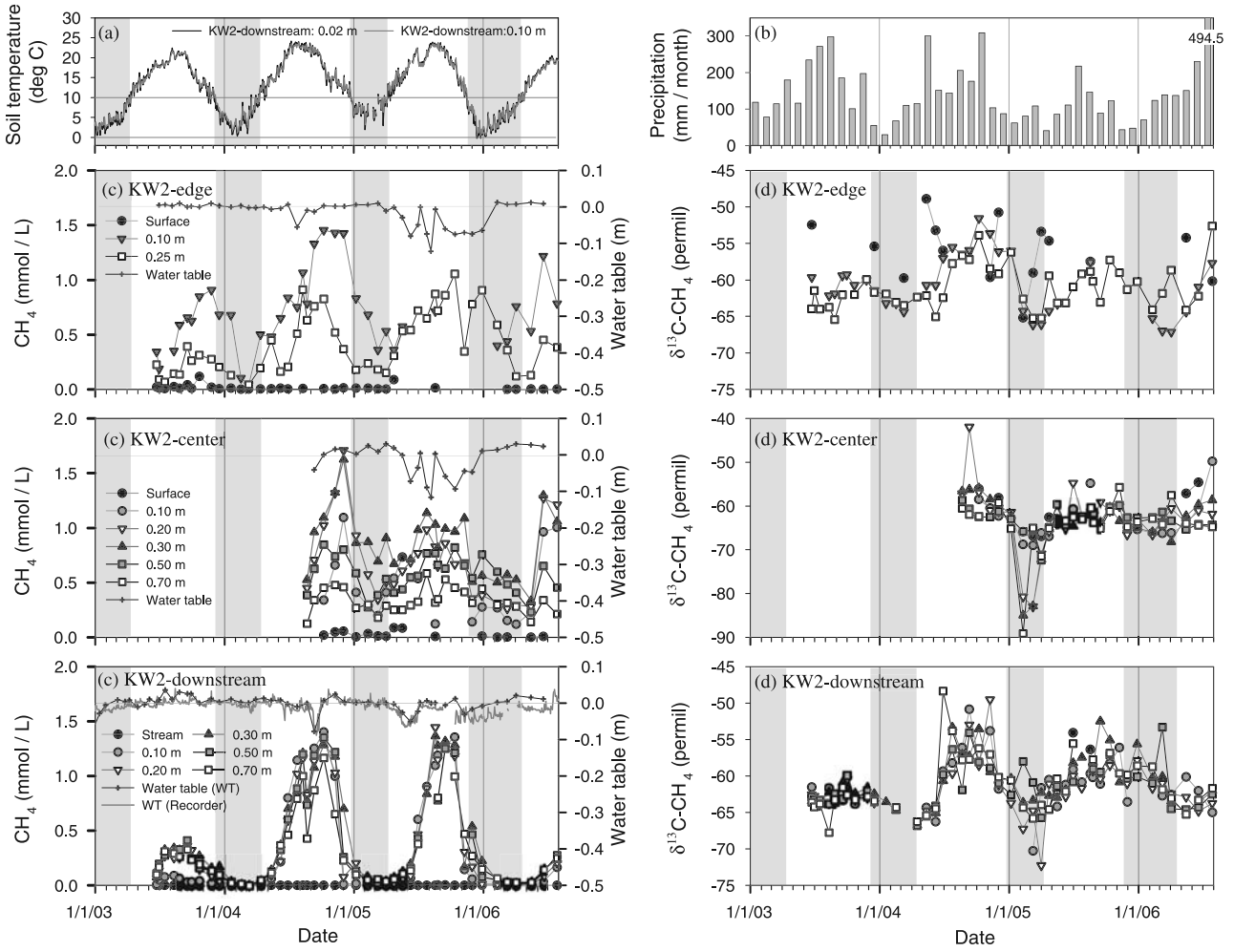

Figure 2. Seasonal variations in (a) soil temperature at KW2-downstream, (b) monthly precipitation measured at the nearby meteorological station, (c) groundwater CH_4 concentration and water table level, and (d) $\delta^{13}C$ of CH_4 in each sampling plot. The shaded areas indicate low temperature (<10°C) periods.

[12] Dissolved CH_4 and CO_2 concentrations were determined within 8 h of sampling by multiple equilibrations with a headspace of ultra high purity (UHP) helium [*McAullife*, 1971]. The headspace was prepared in a vial by replacing sample water with He (>99.999% purity). The vials were vigorously shaken for 2 min to drive gases from the pore water into the headspace. The headspace gas was withdrawn using a gas-tight syringe, and CH_4 concentration was determined using a gas chromatograph (GC; GC-14BPF, Shimadzu, Japan) equipped with a flame ionization detector (FID [*Itoh et al.*, 2007]). For CO_2 concentration measurements, samples of the same gas were collected from the headspace and injected into a GC (GC-8APT, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD [*Itoh et al.*, 2007]). Dissolved CO_2 concentrations in water samples were also measured in situ from June 2003 to August 2005 using a portable pCO_2 meter (CGP-1, DKK-TOA, Japan [*Ohte et al.*, 1995]). GC measurements of dissolved CO_2 concentrations were carried out from February 2005 and confirmed the data obtained by in situ dissolved pCO_2 measurements.

[13] Carbon isotopic compositions of dissolved CH_4 and CO_2 were analyzed using a gas chromatograph/combustion/isotope ratio mass spectrometer (GCCMS) MAT 252 equipped with an HP G1530A system [*Sugimoto*, 1996] at the Center for Ecological Research at Kyoto University. Because of the detection limit, isotopic measurement could not be conducted on samples with low CH_4 or CO_2 concentrations, such as stream and surface water.

2.3. Soil Sampling and Analysis

[14] Mineral and organic soil samples were collected in triplicate in each plot in January 2006. Topsoil and 10–20-cm interval samples underlying the thin litter layer (0–1 cm thick) were collected from the surface to depths of 0.25 m (KW2-edge) or 1.00 m (KW2-center and KW2-downstream). The depth of organic soil was approximately 0.15, 0.40, and 0.50 m in KW2-edge, KW2-center, and KW2-downstream, respectively. Soils were sieved through a 2-mm mesh sieve to remove coarse fragments and then homogenized. The total C and total N concentrations of soil samples were measured using the combustion method [*Bremner*, 1996] in an NC-analyzer (Sumigraph NC-900, Sumigraph Co., Japan).

[15] $\delta^{13}C$ analyses were also carried out on soil samples. The samples were dried in an oven at 40°C for 48 h prior to

Table 2. C and N Content, C/N Ratio, and $\delta^{13}C$ of Litter and Soil at Each Sampling Depth

Plot	Depth (m)	C(%)	N(%)	C/N	$\delta^{13}C$(‰)
Litter		46.4	1.05	45.5	−29.0
KW2-edge	0	5.89	0.226	25.4	−28.0
	0.10	5.72	0.221	26.3	−28.1
	0.25	3.14	0.131	25.0	−28.0
KW2-center	0	10.4	0.547	20.1	−29.0
	0.10	11.3	0.617	18.6	−29.3
	0.20	7.25	0.384	19.2	−29.0
	0.30	2.30	0.114	19.0	−28.5
	0.50	0.65	0.0316	20.4	−27.9
	0.70	1.29	0.0630	19.2	−28.2
KW2-downstream	0	1.73	0.0753	22.2	−28.6
	0.10	2.37	0.0967	24.6	−28.5
	0.20	5.53	0.229	24.3	−28.5
	0.30	6.30	0.280	22.5	−28.5
	0.50	4.27	0.220	19.6	−28.1
	0.70	2.06	0.100	20.7	−28.2

$\delta^{13}C$ analysis. $\delta^{13}C$ was determined using a mass spectrometer (Delta plus XP, Thermo Electron) coupled with an elemental analyzer (Flash EA, Thermo Electron).

3. Results

3.1. C and N Concentrations and $\delta^{13}C$ of Wetland Soils

[16] C and N contents, the C/N ratio, and $\delta^{13}C$ of litter and soil at each sampling depth are shown in Table 2. At KW2-edge and KW2-center, litter accumulates in the surface soils because of slow degradation under wet and anoxic conditions, resulting in higher soil C concentrations at the surface than in the bottom layer. At KW2-center, in particular, the upper soil layer contained much more organic matter than the bottom layer or the other plots. At KW2-downstream, soil C concentration was highest at 0.30 m. At all sampling plots, soil $\delta^{13}C$ values ranged between −29.8 and −28.3‰, and there was no obvious trend in $\delta^{13}C$ with depth or plot (Table 2).

3.2. CH_4 and CO_2 Concentrations and Carbon Isotopic Composition

[17] CH_4 and CO_2 concentrations in groundwater increased with temperature at all three plots (Figures 2c, 3c,

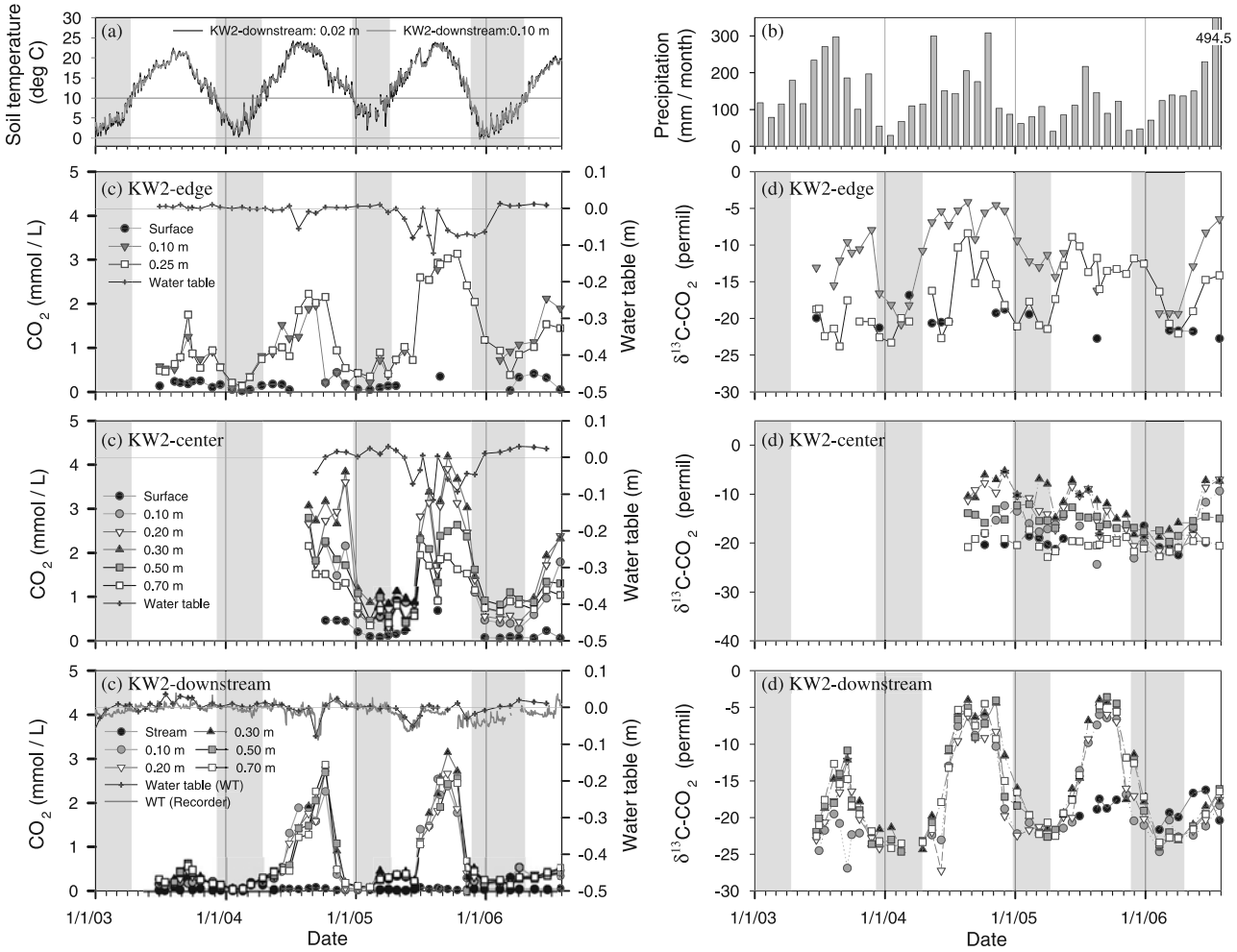

Figure 3. Seasonal variations in (a) soil temperature at KW2-downstream, (b) monthly precipitation measured at the nearby meteorological station, (c) groundwater CO_2 concentration and water table level, and (d) $\delta^{13}C$ of CO_2 in each sampling plot. The shaded areas indicate low temperature (<10°C) periods.

Table 3. Mean, Minimum, and Maximum $\delta^{13}CH_4$ and $\delta^{13}CO_2$[a]

Plot	Depth (m)	Mean CH_4 Concentration (mmol/L)	n	$\delta^{13}C$-CH_4 (‰) Mean	Min	Max	N	Mean CO_2 Concentration (mmol/L)	n	$\delta^{13}C$-CO_2 (‰) Mean	Min	Max	n
KW2-edge	0	0.01	32	−55.4	−65.2	−49.3	14	0.17	29	−19.7	−22.7	−15.4	17
	0.10	0.69	36	−60.3	−67.1	−51.6	32	0.91	35	−11.7	−20.8	−4.1	33
	0.25	0.42	45	−60.8	−65.2	−53.9	43	1.24	44	−17.1	−23.8	−8.4	42
KW2-center	0	0.03	15	−61.7	−66.9	−54.6	10	0.21	17	−19.7	−22.5	−16.5	13
	0.10	0.42	19	−63.7	−69.0	−54.8	18	0.94	17	−17.7	−24.4	−11.7	18
	0.20	0.69	27	−62.8	−83.0	−41.9	26	1.72	27	−13.8	−21.6	−5.6	26
	0.30	0.86	27	−64.1	−85.0	−56.0	26	1.99	27	−12.2	−18.8	−5.3	26
	0.50	0.58	27	−62.9	−72.4	−58.7	26	1.48	27	−15.5	−19.8	−12.1	26
	0.70	0.34	27	−64.0	−89.1	−55.7	24	1.16	26	−20.3	−22.8	−17.3	24
KW2-downstream	0	5.3E-04	46	−55.4	−58.8	−51.8	4	0.05	44	−18.6	−21.7	−16.3	10
	0.10	0.33	46	−60.4	−70.3	−50.8	35	0.64	44	−17.3	−26.9	−5.9	36
	0.20	0.37	46	−61.0	−72.3	−49.5	39	0.66	43	−17.3	−27.2	−5.0	39
	0.30	0.42	46	−60.6	−66.7	−52.5	42	0.73	44	−15.9	−24.5	−4.0	41
	0.50	0.36	43	−60.7	−66.8	−50.8	40	0.62	41	−16.6	−24.6	−3.6	39
	0.70	0.29	43	−60.8	−66.3	−48.4	40	0.60	39	−16.8	−24.1	−4.5	40

[a]Data from June 2003 to June 2006 at KW2-edge and KW2-downstream, and from August 2004 to June 2006 at KW2-center.

and 5). The $\delta^{13}C$ of pore water CH_4 usually increased from summer to autumn at KW2-edge and KW2-downstream. At KW2-center, variation was relatively small in summer (Figure 2d). CH_4 production generally increased with temperature; however, Figures 2a and 2c suggest a time lag between temperature change and methanogenic activity (approximately 1–2 months).

[18] At KW2-edge, CH_4 was higher at 0.10 m than at 0.25 m, except in summer 2005 when the water table dropped (Figure 2c), suggesting that the most reducing conditions were usually at 0.10 m [*Itoh et al.*, 2007]. CO_2 in groundwater increased in high temperature periods and was highest in summer 2005, when the surface soil was drier than usual (Figure 3c). $\delta^{13}C$-CH_4 values at 0.10 m were slightly higher than those at 0.25 m in the hot and wet summer of 2004 and lower in low temperature periods (Figure 2d). In this plot, the maximum $\delta^{13}C$-CO_2 value was −4.1‰ (Table 3). Rapid increases in CH_4 and CO_2 concentrations, $\delta^{13}C$-CH_4, and $\delta^{13}C$-CO_2 in groundwater were observed at the beginning of summer (Figures 2 and 3). This indicates that microbial activity increased rapidly with temperature in this plot.

[19] At KW2-center, CH_4 in groundwater was highest at 0.30 m through the sampling period (Figure 2c and Table 3) and maintained a high level even in low temperature periods (<10°C), when it decreased dramatically in other plots (Figure 2c). In this plot, mean and maximum CO_2 in groundwater were higher than those in the other plots. CO_2 was also highest at 0.30 m, where the soil C concentration was highest (Table 2 and Figure 3c). The range of $\delta^{13}C$-CH_4 values was much larger than in any other plot (Figure 2d and Table 3). That is, we observed lower $\delta^{13}C$-CH_4 values in low temperature periods (e.g., −83.0‰ at 0.30 m depth, 9 March 2005; −85.0‰ at 0.50 m and −89.1‰ at 0.70 m, 9 February 2005; Figure 2d and Table 3) and higher values in high temperature periods (e.g., −41.9‰ at 0.20 m, 10 September 2004), relative to other plots. $\delta^{13}C$-CH_4 was highest at 0.20 m in summer 2004, and no clear vertical trend was seen in other summers (Figure 2d). A temporal increase in $\delta^{13}C$-CO_2 was seen in the high temperature period, but this trend was unclear in the other two plots. Comparisons of the vertical distributions of CH_4, CO_2, and $\delta^{13}C$-CO_2 between the summers of 2004 and 2005 (Figures 2c, 3c, and 3d) all showed convex distributions, peaking at 0.30 m in both summers.

[20] At KW2-downstream, no clear vertical distribution pattern in CH_4 concentration was observed below 0.20 m. Seasonal (low in late winter and high in late summer) and yearly variations in CH_4 were clearer than in the other two plots. Maximum groundwater CH_4 and CO_2 in this plot were much lower in 2003 than in 2004 and 2005, especially at 0.10 m, indicating less microbial activity at low temperatures and high levels of DO in the rainy summer of 2003 (Figures 2c and 3c [*Itoh et al.*, 2007]). Maximum $\delta^{13}C$-CH_4 (Figure 2d) and $\delta^{13}C$-CO_2 (Figure 3d) were also much lower in 2003, especially at 0.10 m. For example, maximum $\delta^{13}C$-CO_2 was much lower in 2003 (−10.9‰ at 0.50 m, 18 September 2003) than in 2004 (−4.0‰ at 0.30 m, 21 August 2004) and 2005 (−3.6‰ at 0.50 m, 15 September 2005). $\delta^{13}C$-CO_2 in groundwater showed clear seasonal changes, ranging from −27 to −4‰, and increased dramatically in high temperature periods (Figure 3d). $\delta^{13}C$-CH_4 showed a similar seasonal change, though not as obvious as $\delta^{13}C$-CO_2 (Figure 2d). In this plot, no obvious vertical difference in $\delta^{13}C$-CH_4 was observed (Figure 2d). $\delta^{13}C$-CH_4 increased dramatically in the summers of 2004 (at all depths) and 2005 (especially at 0.30 m).

4. Discussion

4.1. Effects of Temperature and Hydrology on Changes in CH_4 and CO_2 Dynamics

[21] The measured soil $\delta^{13}C$ at KW2 ranged from −27.9 to −29.3‰ (Table 2). The measured $\delta^{13}C$ of leaves ranges from −24.6 to −28.9‰ in cypress [*Matsuo*, 2003], which is the major vegetation in KEW, and from −33.3 to −32.5‰ in forest floor vegetation (*Eurya japonica*, N. Matsuo, personal communication). CO_2 is produced as a by-product of respiration under both oxic and anoxic conditions in soils. CO_2 produced from the metabolism of lactate is

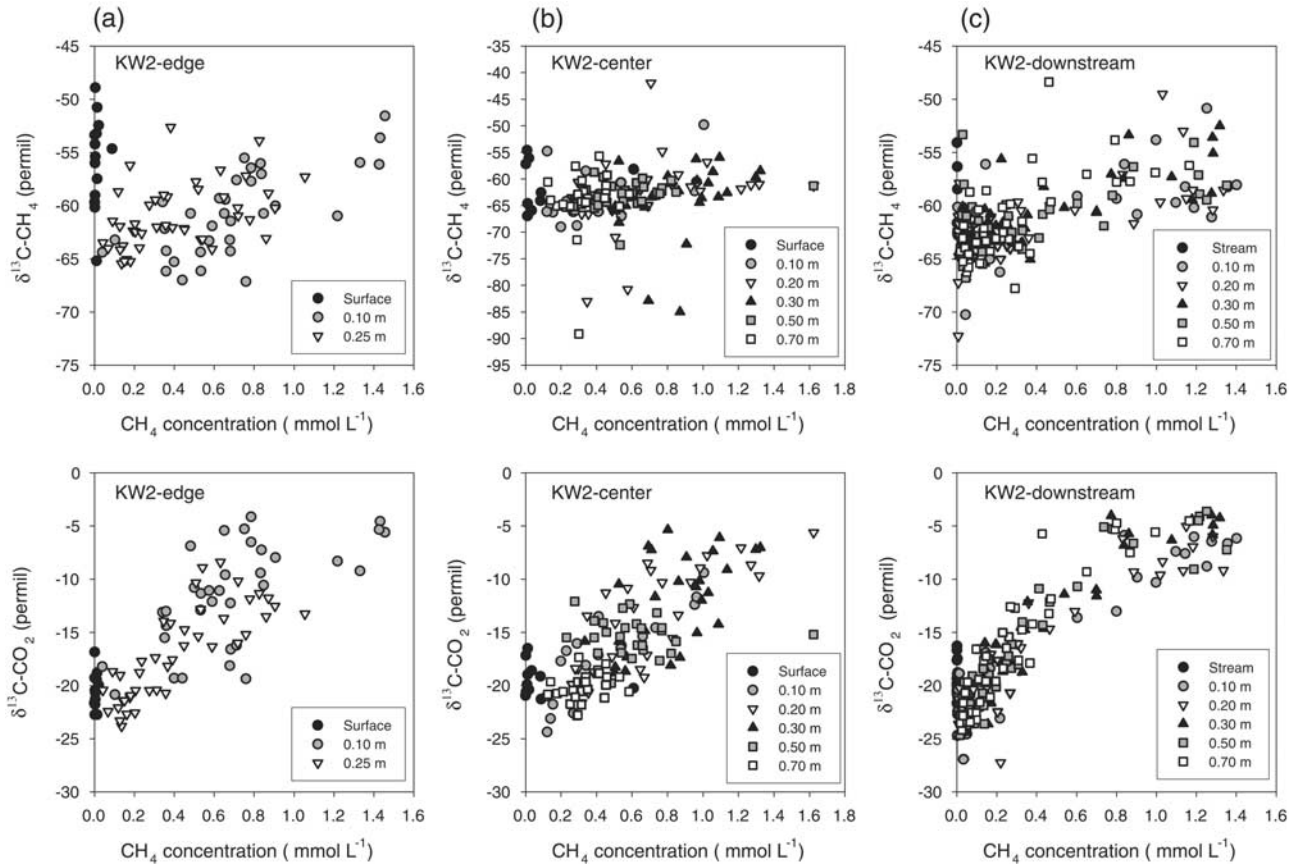

Figure 4. Relationships between CH_4 concentration and $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 at (a) KW2-edge, (b) KW2-center, and (c) KW2-downstream.

depleted in ^{13}C by as much as 26‰ [*Smejkal et al.*, 1971]. Also, CO_2 respired during heterotrophic microbial metabolism is 3.4‰ depleted in ^{13}C relative to the glucose used as the carbon source [*Blair et al.*, 1985]. Negative isotopic enrichment occurs during the decomposition process, whatever the substrate [*Mary et al.*, 1992]. The $\delta^{13}C$ of CO_2 respired by decomposing litter and soil organic matter in KEW is expected to be lower than the $\delta^{13}C$ of soil organic matter (=−29‰ in surface soil at KW2; Table 2), which is similar to the ^{13}C of cypress.

[22] However, we observed much heavier $\delta^{13}C$-CO_2 values in groundwater than in soil, litter, and vegetation. At KW2-edge and KW2-downstream, $\delta^{13}C$-CO_2 dramatically increased in the summer when dissolved CH_4 concentrations were high (Figures 2c and 3d). At KW2-center, $\delta^{13}C$-CO_2 was also much higher than soil $\delta^{13}C$ (Table 3). Processes that might induce such an increase in $\delta^{13}C$-CO_2 are (1) preferential use of $^{12}CO_2$ as a substrate for CH_4 by carbonate reduction or (2) ^{13}C-enriched CO_2 as a by-product of methanogenesis via acetate fermentation under anoxic conditions [*Sugimoto and Wada*, 1993]. CO_2 produced in this manner, i.e., by acetate fermentation, is strongly enriched in ^{13}C and may have a $\delta^{13}C$ value as high as −5‰ [*Charman et al.*, 1994] or +9‰ [*Waldron et al.*, 1999].

[23] The significant positive regression of CH_4 concentration on $\delta^{13}C$-CO_2 (Figures 4 and 5 and Table 4) in all plots suggests that much of the CH_4 produced originates from carbonate reduction. Heavy $\delta^{13}C$-CO_2 from acetate fermentation may also contribute to CO_2 enrichment. The fact that the CO_2 concentration does not decrease in this zone indicates that CO_2 is continuously added by organic remineralization to the pool in the methanogenic zone, although ^{13}C enrichment by methanogenesis does occur. Here, the increase in the proportion of carbonate reduction with temperature is confirmed by an increase in the apparent α in high temperature periods (Figures 6, 7, and 8; discussed below). These results support the idea that carbonate reduction becomes dominant in high temperature periods. As for carbonate (CO_2/H_2) reduction, although we have no data on hydrogen concentrations in groundwater, hydrogen concentration can increase with soil temperature by stimulating litter decomposition [*Sugimoto and Fujita*, 2006]. A less obvious increase in $\delta^{13}C$-CO_2 in the rainy summer of 2003 at KW2-downstream (Figure 3d) was attributed to lower CH_4 production than in a typical summer because of the DO supplied by increased water flow [*Itoh et al.*, 2007]. These results show that preferential use of $^{12}CO_2$ for methanogenesis can drastically change $\delta^{13}C$-CO_2. Although the coefficients were not statistically significant at the deep zone of KW2-center, where CH_4 concentration was high throughout the year (Table 4), our results suggest that CO_2 contributed largely to CH_4 production in our site and residual CO_2 was enriched with ^{13}C during this process.

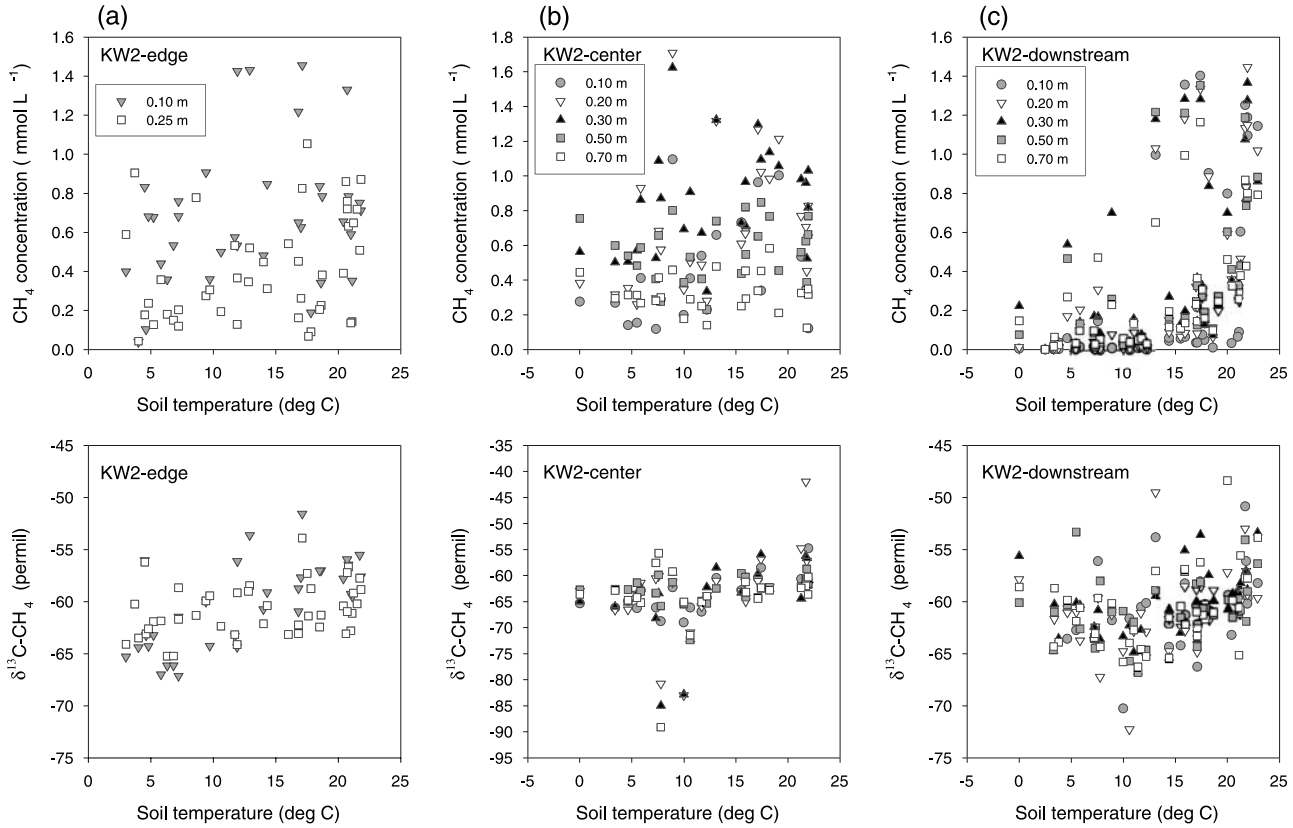

Figure 5. Relationships between soil temperature and CH_4 concentration and $\delta^{13}C$-CH_4 at (a) KW2-edge, (b) KW2-center, and (c) KW2-downstream.

In addition, changes in both temperature and hydrologic conditions (rainfall and the water table) played important roles in the variations in CH_4 and CO_2 concentrations and $\delta^{13}C$-CO_2. However, acetate fermentation must affect CH_4 production, and we consider the effects of acetate contributions to methanogenesis in the next section.

4.2. Acetate Contribution Determined by Isotope Mass Balance

[24] Similar seasonal variations in $\delta^{13}C$ of CO_2 and CH_4 indicated that CO_2 contributes much as a substrate for CH_4 production. However, $\delta^{13}C$-CH_4 varied seasonally and fluctuated more wildly than $\delta^{13}C$-CO_2 at KW2-downstream (Figures 2d and 3d). Remarkably high $\delta^{13}C$-CH_4 values were occasionally observed in summer (e.g., KW2-downstream: −48.4‰ on 28 June 2004 at 0.70 m; −50.8‰ on 10 September 2004 at 0.10 m; −49.5‰ on 8 November 2004 at 0.20 m; and −52.5‰ on 15 September 2005 at 0.30 m; Figure 2d) when high CH_4 production occurred. Also at KW2-edge, heavy $\delta^{13}C$-CH_4 values were observed in summer (i.e., −51.6‰ at 0.10 m on 7 October 2004 and −57.3‰ at 0.25 m on 12 October 2005). The crossplots of $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 are shown in Figures 6a–6c, with the apparent fractionation between CO_2 and CH_4 (α) calculated by the

Table 4. Results of Regression Analyses Between CH_4 Concentration and $\delta^{13}CH_4$ or $\delta^{13}CO_2$ at Each Sampling Depth[a]

		$\delta^{13}CH_4$				$\delta^{13}CO_2$			
Plot	Depth (m)	n	F	p	r^2	n	F	p	r^2
KW2-edge	0.10	32	21.7	<0.001	0.41	33	22.7	<0.001	0.41
	0.25	42	5.67	<0.05	0.12	41	75.3	<0.001	0.65
KW2-center	0.10	18	4.81	<0.05	0.22	18	46.1	<0.001	0.73
	0.20	26	2.35	0.137	0.09	26	35.6	<0.001	0.59
	0.30	26	2.13	0.157	0.08	26	18.8	<0.01	0.43
	0.50	26	2.91	0.100	0.10	26	0.164	0.689	0.01
	0.70	24	0.479	0.495	0.02	24	2.06	0.164	0.08
KW2-downstream	0.10	33	21.7	<0.001	0.40	36	352	<0.001	0.91
	0.20	39	25.3	<0.001	0.40	38	205	<0.001	0.85
	0.30	42	60.9	<0.001	0.60	41	320	<0.001	0.89
	0.50	39	19.5	<0.001	0.34	39	229	<0.001	0.86
	0.70	39	27.5	<0.001	0.42	40	191	<0.001	0.83

[a]Values of F and p are observed variance ratios and probabilities in the analyses, respectively.

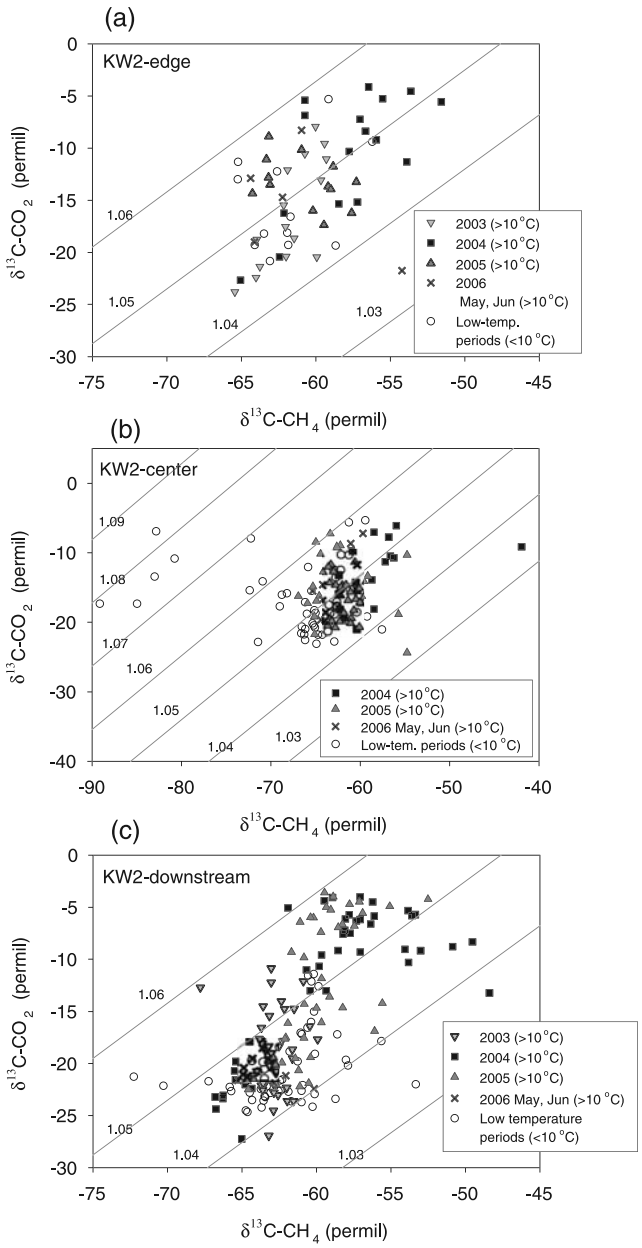

Figure 6. Crossplots of $\delta^{13}C$ data from CH_4 and CO_2 in groundwater at (a) KW2-edge, (b) KW2-center, and (c) KW2-downstream. The dashed lines give the apparent fractionation between CO_2 and CH_4 (α) calculated by the ratio of $(\delta^{13}C\text{-}CO_2 + 10^3)/(\delta^{13}C\text{-}CH_4 + 10^3)$.

ratio $(\delta^{13}C\text{-}CO_2 + 1000)/(\delta^{13}C\text{-}CH_4 + 1000)$. From this, the apparent α was widely distributed from 1.032 to 1.083 and was usually between 1.04 and 1.06. This range is lower than reported values for ^{13}C fractionation during CO_2 reduction to CH_4 (α_c = 1.06–1.07) according to a review of experimentally determined α_c of natural wetlands [*Conrad*, 2005]. Here,

$$\alpha_C = \frac{(\delta^{13}CO_2 + 1000)}{(\delta^{13}CH_{4(CO2)} + 1000)} \quad (1)$$

where $\delta^{13}CO_2$ is the $\delta^{13}C$ value of the CO_2 pool at the sampling time and $\delta^{13}CH_{4(CO2)}$ is the $\delta^{13}C$ value of CH_4 from CO_2.

[25] Our results indicate that acetate fermentation, which has a smaller isotopic fractionation than carbonate reduction, affected the change in apparent α in our wetland. Here, we calculate the acetate contribution (F_{Ac}) to CH_4 production by means of isotope mass balance [*Sugimoto and Wada*, 1993]. In this calculation, only two substrates, CO_2 and acetate, are considered for methanogenesis, because CH_4 from other substrates such as methanol and trimethylamine has so far not been found to play a major role in freshwater environments [*Lovley and Klug*, 1983b; *Conrad and Claus*, 2005]. The fractional Ac contribution is expressed by F_{Ac} as:

$$F_{Ac} = \frac{CH_4 \text{ from acetate}}{CH_4 \text{ from } CO_2 + CH_4 \text{ from acetate}} \quad (2)$$

[26] The isotope mass balance for produced CH_4 is expressed by the following equation:

$$\delta^{13}CH_{4(Ac)}F_{Ac} + \delta^{13}CH_{4(CO2)}(1 - F_{Ac}) = \delta^{13}CH_4 \quad (3)$$

where $\delta^{13}CH_{4(Ac)}$ and $\delta^{13}CH_{4(CO2)}$ are the $\delta^{13}C$ values of CH_4 produced from acetate and CO_2, respectively, and $\delta^{13}CH_4$ is that of CH_4 produced during the indicated period. When the $\delta^{13}C$ values of CH_4, $\delta^{13}CH_{4\ (Ac)}$, and $\delta^{13}CH_{4\ (CO2)}$ are obtained, F_{Ac} can be calculated from this equation. $\delta^{13}CH_{4\ (CO2)}$ was calculated using equation (1).

[27] Because we have no data on $\delta^{13}C$ of acetate and CH_4 from acetate, we used possible $\delta^{13}C$ values of CH_4 from acetate ranging from −44 to −27‰, considering the values obtained from previous studies [e.g., *Sugimoto and Wada*, 1993; *Avery et al.*, 1999; *Nakagawa et al.*, 2002], and $\delta^{13}C$ data from soil collected from KW2 (Table 2). During acetate fermentation, CH_4 is produced primarily from the methyl carbon of acetate [*Pine and Barker*, 1956; *Krzycki et al.*, 1982]. *Sugimoto and Wada* [1993] incubated Japanese rice paddy field soil ($\delta^{13}C$ = −26.5‰) with BES (a methanogenesis inhibitor) and measured the $\delta^{13}C$ of both methyl carbon and carboxyl carbon of acetate. The $\delta^{13}C$ of methyl carbon ranged from −36 to −30‰, which was lower than that of carboxyl carbon (−21 to −15‰). From this and the $\delta^{13}C$ of our wetland soils (approximately −29‰), we assume $\delta^{13}CH_{4\ (Ac)}$ = −35‰. We must also assume values for the ^{13}C fractionation coefficient during CO_2 reduction to CH_4 (α_c). When assuming the α_c value, the temperature dependence of α_c [e.g., *Whiticar et al.*, 1986; *Whiticar*, 1999; *Conrad*, 2005] is considered. *Blair et al.* [1993] found that α_c in marine sediment decreased with increasing temperature according to:

$$\ln \alpha_C = (23.0/T) - 0.022 \quad (4)$$

[28] A similar relationship was found for methanogenesis cultures [*Botz et al.*, 1996], $\ln \alpha_c = (29/T) - 0.030$. We used the slope of equation (4) with the soil temperature range of KEW (0°C to 23°C at a depth of 0.10 m at KW2-downstream). As a first approximation, we assumed $\ln \alpha_c = (23/T) - 0.022$ according to the value of 1.06–1.07 based on a review of

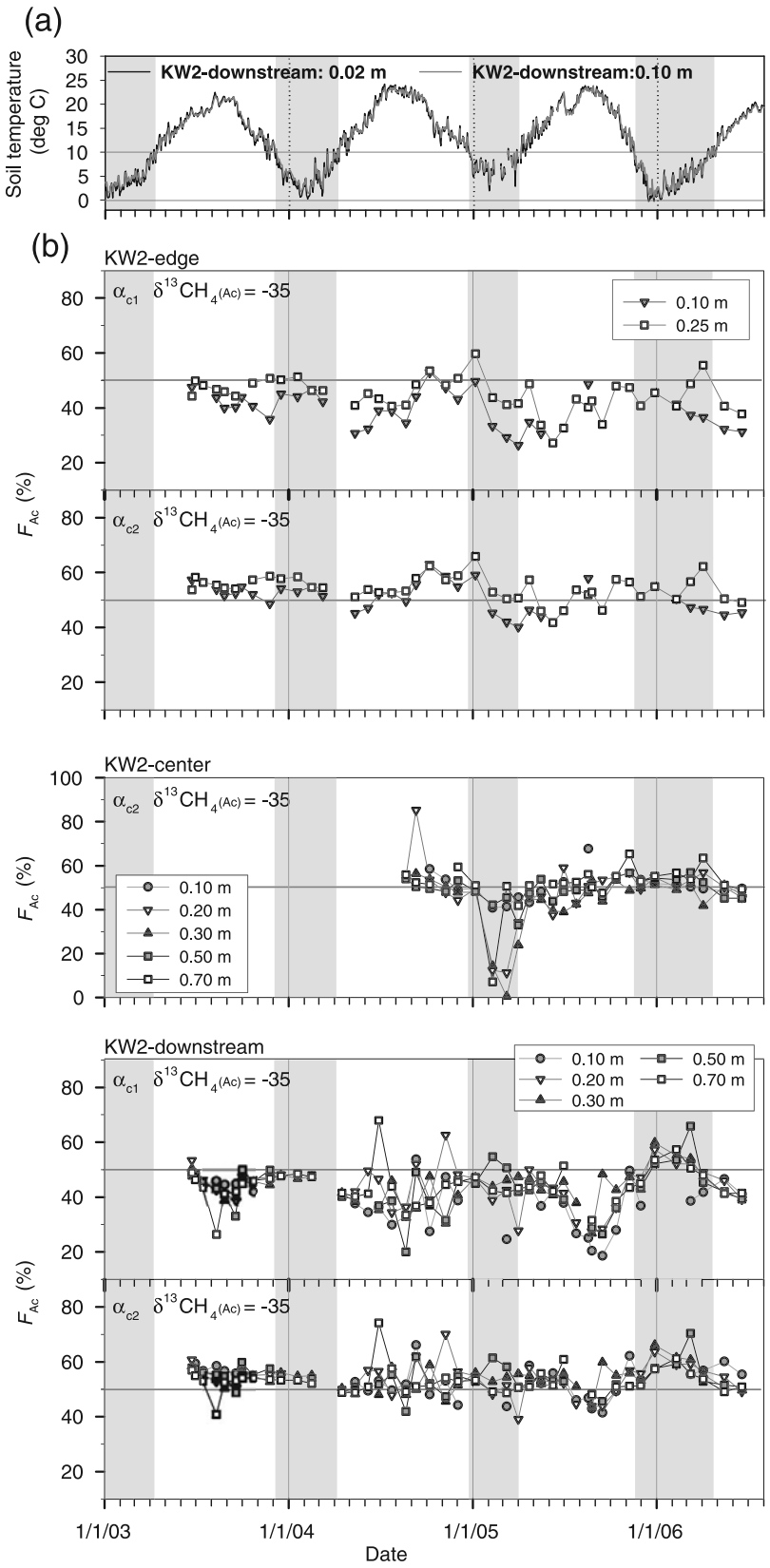

Figure 7. Seasonal variation in (a) soil temperature and (b) F_{Ac} (acetate contribution to methanogenesis) in each sampling plot. The shaded areas indicate low temperature (<10°C) periods.

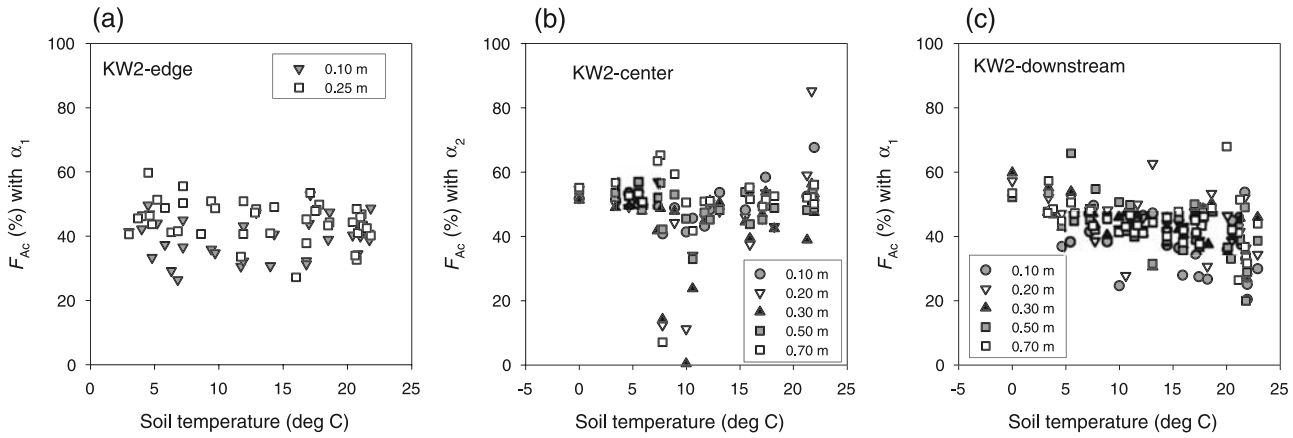

Figure 8. Relationships between soil temperature and F_{Ac} at (a) KW2-edge, (b) KW2-center, and (c) KW2-downstream.

experimentally determined α_c of natural wetlands [*Conrad*, 2005], resulting in a range of 1.068 at 23°C to 1.075 at 0°C (α_{c1}) for α_c. Next, if the F_{Ac} result was negative, we assumed a larger α_c value in order to obtain positive F_{Ac} results (α_{c2}).

[29] Figure 7b shows the seasonal variation of F_{Ac} calculated from the above equations, assuming α_{c1} and $\delta^{13}CH_{4\ (Ac)} = -35‰$ for KW2-edge and KW2-downstream. Table 5 shows the possible ranges of F_{Ac} with varying parameters. For KW2-center, however, calculated F_{Ac} values using α_{c1} in winter 2004 were negative for depths of 0.20 and 0.30 m regardless of the $\delta^{13}CH_{4\ (Ac)}$ value (Table 5). Therefore, we assumed α_{c2} [$\ln \alpha_{c2} = (23/T) - 0.0015$], resulting in a range of 1.079 at 23°C to 1.086 at 0°C, and used these values to calculate Ac for the three plots (Figure 7b and Table 5).

[30] Larger F_{Ac} values result from larger α_c (at most 14.5% given the same $\delta^{13}CH_{4\ (Ac)}$) and $\delta^{13}CH_{4\ (Ac)}$ (at most 18.5% given the same α_c). Also, smaller α_c and larger $\delta^{13}CH_{4\ (Ac)}$ values result in wider ranges of F_{Ac} (Figure 7b and Table 5). This indicates that the setting of these values changes the result significantly. However, the seasonal trend of F_{Ac} was characteristic in each plot and did not change with changes to the parameters. As mentioned above, for purposes of discussion, here we used the results based on an appropriate $\delta^{13}CH_{4\ (Ac)}$ value for KEW of −35‰ [*Sugimoto and Wada*, 1993]. In addition, some attention should be paid to the expected systematic change in $\delta^{13}CH_{4\ (Ac)}$ with temperature, although the degree of change is unknown because this has not been studied in detail. The range of possible $\delta^{13}CH_{4\ (Ac)}$ is most

Table 5. Mean, Minimum, and Maximum Ac Contributions Calculated Using Equation (3)[a]

Plot	Depth (m)	α_c	Ac Contribution (%) $\delta^{13}CH_4$ (Ac) = −27 Mean (Min. – Max.)	Ac Contribution (%) $\delta^{13}CH_4$ (Ac) = −35 Mean (Min. – Max.)	Ac Contribution (%) $\delta^{13}CH_4$ (Ac) = −44 Mean (Min. – Max.)
Edge	0.10	α_{c1}	33.1 (22.3 – 43.1)	39.5 (26.5 – 52.9)	50.6 (33.6 – 71.1)
	0.25	α_{c1}	38.2 (22.5 – 51.9)	44.7 (27.2 – 59.8)	55.4 (35.5 – 72.1)
	0.10	α_{c2}	44.1 (34.8 – 53.6)	51.0 (40.1 – 63.1)	62.0 (48.5 – 78.9)
	0.25	α_{c2}	47.5 (35.8 – 58.4)	54.2 (41.8 – 65.9)	64.6 (51.3 – 77.1)
Center	0.10	α_{c1}	34.6 (24.7 – 53.4)	40.3 (28.9 – 61.7)	49.6 (35.6 – 74.7)
	0.20	α_{c1}	30.9 (*6.8* – 66.9)	36.6 (*8.1* – 81.3)	46.2 (*10.3* – *107*)
	0.30	α_{c1}	26.4 (*20.5* – 37.7)	31.3 (*24.7* – 45.4)	39.8 (*32.3* – 59.1)
	0.50	α_{c1}	32.9 (16.2 – 42.3)	38.7 (19.0 – 48.9)	48.2 (23.6 – 59.2)
	0.70	α_{c1}	38.3 (27.2 – 50.6)	44.4 (31.3 – 58.7)	54.1 (37.7 – 71.5)
	0.10	α_{c2}	44.2 (35.8 – 59.9)	50.3 (40.8 – 67.7)	59.3 (49.0 – 75.1)
	0.20	α_{c2}	41.9 (9.8 – 72.9)	48.2 (11.2 – 85.3)	58.0 (13.5 – *105*)
	0.30	α_{c2}	38.3 (0.3 – 48.3)	44.2 (0.4 – 56.3)	53.4 (0.5 – 69.1)
	0.50	α_{c2}	43.1 (28.8 – 50.3)	49.4 (32.9 – 56.9)	59.0 (39.3 – 67.2)
	0.70	α_{c2}	45.3 (6.2 – 57.6)	51.4 (7.1 – 65.3)	60.5 (8.4 – 76.9)
Downstream	0.10	α_{c1}	31.9 (14.9 – 43.6)	38.0 (18.6 – 53.8)	48.6 (25.9 – 72.9)
	0.20	α_{c1}	37.7 (22.2 – 52.0)	44.1 (27.5 – 62.6)	54.8 (33.7 – 81.5)
	0.30	α_{c1}	37.3 (21.5 – 51.7)	43.8 (26.8 – 59.8)	54.8 (37.0 – 72.5)
	0.50	α_{c1}	36.7 (16.2 – 57.3)	43.0 (20.0 – 65.9)	53.4 (27.3 – 79.1)
	0.70	α_{c1}	37.8 (22.1 – 57.0)	44.3 (26.4 – 67.9)	55.1 (33.9 – 86.6)
	0.10	α_{c2}	46.4 (35.2 – 56.5)	53.2 (41.5 – 66.1)	60.9 (42.4 – 76.4)
	0.20	α_{c2}	47.1 (34.5 – 60.2)	53.8 (39.1 – 70.1)	64.2 (45.8 – 86.0)
	0.30	α_{c2}	47.0 (36.7 – 58.4)	53.8 (43.6 – 66.1)	64.5 (55.4 – 77.6)
	0.50	α_{c2}	46.4 (32.0 – 63.0)	53.1 (38.0 – 71.0)	63.4 (47.9 – 82.8)
	0.70	α_{c2}	46.2 (35.6 – 64.2)	53.0 (41.2 – 74.1)	63.5 (49.9 – 89.7)

[a]Data obtained from June 2003 to June 2006 at KW2-edge and KW2-downstream, and from August 2004 to June 2006 at KW2-center.

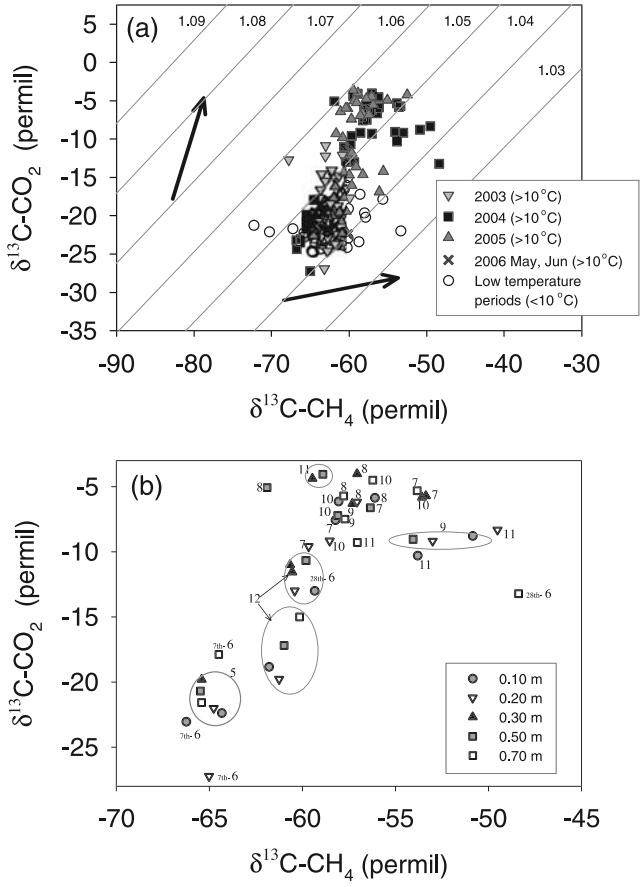

Figure 9. Crossplot of $\delta^{13}C$ data from CH_4 and CO_2 in groundwater at KW2-downstream. The dashed lines give the apparent fractionation between CO_2 and CH_4. Arrows indicate temporal change in $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 during an incubation experiment using rice paddy soil at different temperatures [*Fey et al.*, 2004]. Arrow 1 is from the results of experiments conducted under the condition that CO_2 reduction solely contributed to methanogenesis. Arrow 2 is provided under the condition that acetate fermentation was the dominant form of methanogenesis. Figure 9b shows the data at KW2-downstream in summer 2004 labeled by sampling month.

likely within the ranges reported in Table 5. Therefore, in our discussion, this effect would be covered by the ranges used for calculating F_{Ac} with various parameters.

[31] Mean acetate contributions during sampling periods varied between 30 and 44% with α_{c1} and 44 and 54% with α_{c2} (Figure 7b and Table 5). As mentioned above, the calculated F_{Ac} at KW2-center fell below 0% in winter 2004/2005 with α_{c1}, indicating that α_{c2} was more appropriate for this plot. At KW2-edge, F_{Ac} increased in late summer 2004 and decreased from winter to summer 2005 (Figure 7b). At KW2-center, F_{Ac} was usually stable expect in winter 2004/2005 (≈0%, Figure 7b). At KW2-downstream, the seasonal trend was a lower F_{Ac} in high temperature periods (40.3% with α_{c1} and 52.7% with α_{c2}, averaged from 0.10 m to 0.70 m) and higher F_{Ac} in low temperature periods (45.3% with α_{c1} and 54.4% with α_{c2}); however, F_{Ac} showed episodic increases especially in summer 2004 when the most reducing conditions were established (Figure 9b) [*Itoh et al.*, 2007]. These episodic and short-lived increases suggest that acetate is easily exhausted, probably due to its availability. This episodic increase in F_{Ac} was supported by various results. First, incubation experiments showed that active consumption of added acetate induces a rapid increase in $\delta^{13}C$-CH_4 [*Sugimoto and Wada*, 1993]; second, field observations showed rapid decreases in acetate with increases in temperature [*Shannon and White*, 1996; *Avery et al.*, 1999]. Third, pore water acetate concentrations increased during transition phases between acetate consumption by SO_4^{2-} reducers and methanogens [*Alperin et al.*, 1992; *Sugimoto and Wada*, 1993], suggesting a high availability of acetate. On the other hand, F_{Ac} clearly decreased at KW2-edge and downstream in the dry summer of 2005.

[32] Figure 9a shows a crossplot of $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2, with arrows indicating temporal changes in $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2, from an incubation experiment of rice paddy soil at different temperatures [*Fey et al.*, 2004]. Using ^{14}C-labeled bicarbonate, the incubation experiment found CH_4 production to be solely the result of CO_2 reduction at high temperature (50°C) [*Fey and Conrad*, 2000; *Fey et al.*, 2001]. Arrow 1 is provided from the results of experiments conducted under the same conditions, suggesting that α_c = 1.073 [*Fey et al.*, 2004]. The results of *Fey et al.* [2004] also suggest that acetate with heavy $\delta^{13}C$ initially increased and then decreased with increases in CH_4 concentration and $\delta^{13}C$-CH_4 at temperatures of 10–37°C, hence arrow 2. This suggests that when acetate fermentation is dominant, only $\delta^{13}C$-CH_4 becomes heavier. *Chan et al.* [2005] also showed larger α_c, i.e., 1.0864–1.0885 and 1.0811–1.0892, for lake sediment cores sampled in May and August, respectively, by using an inhibitor of acetotrophic methanogenesis (CH_3F). The α_c values shown by *Chan et al.* [2005] are much larger than the apparent α in the present study. Figure 9b shows the summer 2004 data labeled by sampling month. In summer 2004, when CH_4 production dramatically increased, both $\delta^{13}C$-CO_2 and $\delta^{13}C$-CH_4 increased simultaneously in early summer, followed by a sharp increase only in $\delta^{13}C$-CH_4. According to the results of *Fey et al.* [2004], the simultaneous increase in $\delta^{13}C$-CO_2 and $\delta^{13}C$-CH_4 indicates that CO_2 reduction usually is dominant in periods of high temperature, and the contribution from acetate fermentation is variable. This is shown in the linear relationship between $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2, composed of arrows 1 and 2 in Figure 9a. This increase in the proportion of carbonate reduction is also suggested by the increase in apparent α in this season. The outliers (high $\delta^{13}C$-CH_4) from this linear relationship suggest the sporadic and short-lived increase in acetate fermentation (shown as arrow 2 in Figure 9a). It is unlikely that increases in $\delta^{13}C$-CH_4 due to re-oxidation of CH_4 [*Roslev and King*, 1994, 1996] occurred in this period under low DO conditions [*Itoh et al.*, 2007]. Furthermore, only two of nine values with $\delta^{13}CH_4$ above −55‰ are from 0.10 m (CH_4 oxidation may be expected to occur because oxidation is usually observed from the soil surface downward), while during the same period, high CH_4 concentrations were measured up to that depth. Thus, our results suggest that the unusually high $\delta^{13}C$-CH_4 values observed in high temperature periods were due to increases in acetate fermentation under high soil temperature (Figures 8b and 8c). This implies that acetate

fermentation requires more time to be activated than does CO_2 reduction, in agreement with *Vogels et al.* [1988], who showed that doubling times for acetoclastic methanogens were longer than those for CO_2-reducing methanogens.

[33] In addition to the effect of increased temperature, episodic increases in F_{Ac} were also observed in low temperature periods (e.g., KW2-downstream, 0.50 m: −53.3‰, 9 March 2006), implying that there is another factor influencing F_{Ac}, such as supply of acetate or competition for acetate with another acetate utilizer, e.g., sulfate-reducing bacteria (SRB) that are active under almost the same reducing conditions as methanogenesis. SRB may compete for acetate with acetoclastic methanogens under mildly reducing conditions (with some SO_4^{2-} [e.g., *Winfrey and Zeikus*, 1977; *Schönheit et al.*, 1982]). This phenomenon is usually observed in marine sediments, but can also occur in freshwater sediments; SRB can only outcompete methanogenesis when sulfate concentrations are increased to 60 μM [*Lovley and Klug*, 1983a]. In our wetland, the SO_4^{2-} concentration was usually low in summer at all plots (≈0 [*Itoh et al.*, 2007]). In particular, SO_4^{2-} was lowest at all plots in the hot and wet season of 2004. This agrees with the increase in CH_4 production. Also, SO_4^{2-} was higher in rainy (2003; ≈50 μM at 0.10 m) and dry (2005 at 0.50 m) seasons; under such conditions, the activity of SRB may be higher, thereby reducing acetate fermentation. Under highly reducing conditions, SRB activity becomes limited by a decrease in available sulfate; thus, acetate may become available for methanogenesis under highly reducing summer conditions.

[34] F_{Ac} decreased more in the dry summer of 2005 than in 2004 at KW2-downstream (Figure 7b; summer to fall 2005), indicating that acetate fermentation may be inhibited by a decrease in acetate supply with low water transport in a low precipitation summer. In fact, the water table in the entire KW2 area was much lower in summer 2005 than in 2004 (Figure 2c).

[35] The relationship between $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 was unclear at KW2-center (Figure 6b), where strong reducing conditions were maintained during the year. A low level of water exchange with streams indicated by a small hydraulic head [*Itoh et al.*, 2007] and high soil C concentration (Table 2) in this plot may affect the stable contribution of acetate fermentation.

[36] *Conrad et al.* [1989] and *Chin and Conrad* [1995] showed that the contribution of carbonate reduction decreased when temperature decreased (from 30°C to 10°C) and that CH_4 is mainly produced from acetate. Our results indicating a higher acetate contribution in periods of low temperature agree with their report. However, much lower levels of $\delta^{13}C$-CH_4 (and therefore, calculated F_{Ac}) in deeper soil were observed only in the mild winter of 2004/2005 (Figure 2d). The reported range of $\delta^{13}C$-CH_4 from biogenic sources is −41 to −86‰ [*Quay et al.*, 1988] and that from peatlands is −46‰ [*Martens et al.*, 1992] to −83‰ [*Lansdown et al.*, 1992]. The low $\delta^{13}C$ values observed here usually fell in these ranges; however, we do not know why such low values were observed only in the winter of 2004/2005. This winter was warmer than normal, especially in December (monthly average air temperature at meteorological station was 6.3°C; average value from 1996 to 2005 was 5.1°C). In addition, such a large decrease in $\delta^{13}C$-CH_4 was not observed in the much colder winter of 2005/2006 (Figure 2a), suggesting that some factor other than temperature affects this phenomenon. Although statistical analysis showed that hydrological conditions such as rainfall [including the antecedent precipitation index (API)], runoff, and water table are not directly related to this phenomenon (data not shown), we assume that acetate becomes exhausted under conditions of high methanogenic activity, even in early winter 2004, because CH_4 production remained much higher from late summer to autumn 2004 than in other years (Figure 2b). This may have induced the large depletion of $\delta^{13}C$-CH_4 and F_{Ac}, especially at KW2-center where water exchange is slow.

4.3. Relationship Between Changes in Contribution of Acetate Fermentation and CH_4 Flux

[37] At KW2-downstream, the patterns of $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 differed each summer (Figures 2d and 3d) depending on reducing conditions controlled by precipitation, runoff, water table, and thus DO (Table 1 [*Itoh et al.*, 2007]). As described above, based on isotope signatures, the calculated contribution of acetate fermentation usually decreased in high temperature periods; however, it episodically increased under the highly reducing conditions in summer 2004 (Figures 7b and 9b). *Itoh et al.* [2007] reported a dramatic increase in CH_4 emission from the soil surface with increased CH_4 concentration in the surface groundwater in summer 2004. These observations suggest that CH_4 emission from the soil surface results from increased CH_4 production and accumulation in soil. The activation of acetate fermentation under highly reducing conditions probably contributed to this large CH_4 emission. A similar pattern was observed in KW2-edge in summer 2004. These results are consistent with previous observations of high acetate concentrations during early spring, followed by a large CH_4 flux, and then a sharp decrease in acetate concentration in freshwater peatlands in southern Michigan, USA [*Shannon and White*, 1996].

[38] Our results indicate that detecting increases in acetate fermentation using isotopic signatures can provide information on changes in CH_4 production and emission in summers with different redox conditions. Large variations in CH_4 production pathways, as described above, are probably more applicable in regions with large variations in temperature and precipitation, such as the Asian monsoon region. In such regions, the methanogenic pathway can vary widely in spatial and temporal scale with variations in redox conditions. Our observations indicate that the amount of acetate available for methanogenesis is controlled by supply, and therefore both water movement and microbial consumption are important factors.

[39] Schematics of the effects of changes in environmental conditions (e.g., precipitation, water table, and runoff) on CH_4 dynamics (formation pathways, groundwater concentrations, and emissions from soil surfaces) in summer are presented in Figure 10. Under "low temperature" and "high water table" conditions (e.g., the rainy summer of 2003 at our study site), less-reduced conditions than normal for summer were maintained by (1) increased oxygen supply from more frequent incoming water due to high runoff and precipitation and (2) low oxygen consumption due to low microbial activity. In such years, the acetate contribution to CH_4 production is stable and methanogenic activity from

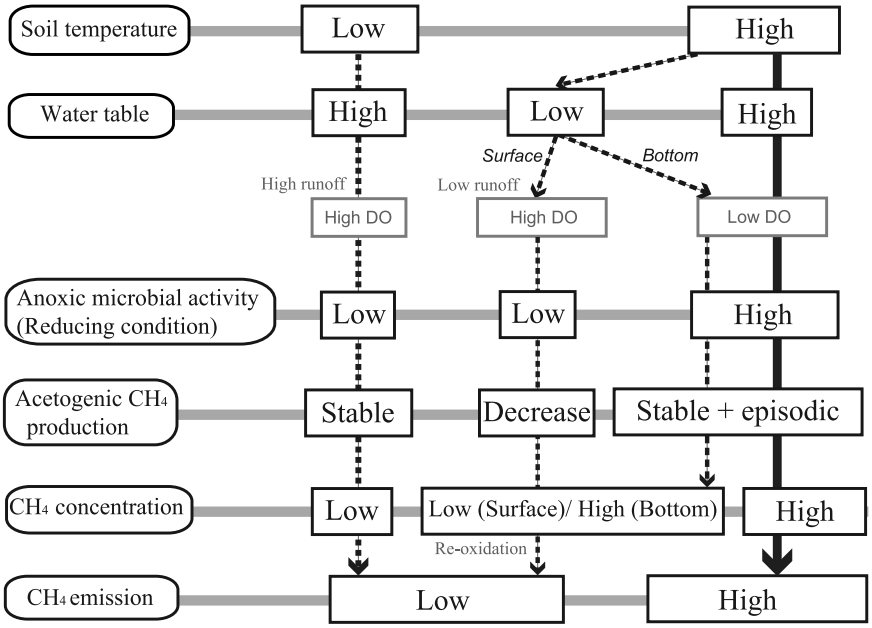

Figure 10. Schematics of the effects of environmental changes on CH_4 dynamics in summer. The thickness of an arrow indicates its degree of contribution.

acetate is low. The amount of CH_4 produced and retained in groundwater is also low; consequently, CH_4 emissions from the soil surface are small (Figure 10).

[40] In a highly reducing summer, under conditions of "high temperature" and "high water table" (e.g., 2004 at our study site), CH_4 production from both carbonate reduction and acetate fermentation becomes active, and the proportion of carbonate reduction is generally increased under high CO_2 concentrations. In addition, acetate fermentation episodically increases under high microbial activity, resulting in heavier ^{13}C-CH_4. A large quantity of CH_4 is retained in surface soils and contributes to high CH_4 emissions from the soil surface.

[41] Under "high temperature" and "low water table" conditions that usually occur in low-precipitation summers (e.g., 2005 at our study site), water table depletion induces the formation of an aerobic (oxic) layer in surface soils. On the other hand, highly reducing conditions form in the bottom layer because of the decreased oxygen levels caused by the reduced water movement [*Itoh et al.*, 2007]. The proportion of acetate fermentation decreases, probably because of low water movement under such conditions. CH_4 emissions from the soil surface are suppressed because of low anoxic microbial activity in the aerobic surface layer. Re-oxidation of CH_4 may occur in this layer. Our results suggest that changes in the CH_4 production pathway are influenced by changes in hydrologically controlled redox conditions and, consequentially, affect emissions from the soil surface.

5. Conclusions

[42] Our results show that changes in environmental conditions, especially hydrological conditions (water table and temperature), affect the CH_4 production pathways in riparian wetlands in temperate forests because hydrological conditions control the spatial and temporal variation in redox conditions. Under "high water table" and "low temperature" conditions (rainy summers with high levels of runoff), high levels of DO create more oxic conditions, and the proportion of carbonate reduction and acetate fermentation is stable and activity levels are low. In contrast, in a highly reducing summer with "high water table" and "high temperature" conditions, the proportion of carbonate reduction increases with soil temperature, with episodic increases in acetate fermentation. In such a period, the decreased SRB activity associated with limited SO_4^{2-} levels can also induce acetate fermentation. This induces higher CH_4 production and CH_4 accumulation in soil and, consequently, high CH_4 emissions from the soil surface. In drier summers with "low water table" and "high temperature" conditions, the contribution of acetate fermentation is lower probably due to oxic conditions and decreased acetate supply. Acetate fermentation also decreases sharply in winter following a highly reducing summer. These patterns indicate that the amount of acetate available for methanogenesis is controlled by supply, and both water movement and microbial consumption are important factors regulating this supply.

[43] The results of this study demonstrate the effects of hydrological changes in wetlands on changes in CH_4 production pathways. Our findings indicate the importance of considering hydrologic effects when assessing CH_4 production in wetlands where redox conditions vary on shorter temporal and smaller spatial scales. In addition, our results that acetate fermentation was highly variable compared to carbonate reduction suggest that a more detailed understanding of what processes affect acetate supply and consumption is required.

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